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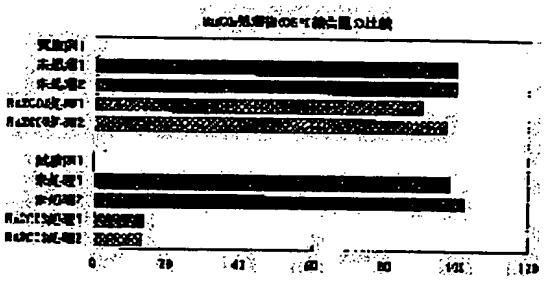
(54) DRUG-ENCAPSULATING INORGANIC MICROPARTICLE, MANUFACTURING METHOD THEREOF AND PREPARATION COMPRISING DRUG-ENCAPSULATING INORGANIC MICROPARTICLE

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a nonirritating drug-encapsulating inorganic microparticle, which is easy to prepare as a preparation, is applicable to many medicinal proteins, low-molecular weight compounds and genes, can stabilize the medicinal proteins, low-molecular weight compounds and genes, and has a good sustained releasability and a good targeting effect, and to also provide a manufacturing method thereof and a preparation



comprising the drug-encapsulating inorganic microparticle.
SOLUTION: The drug-encapsulating inorganic microparticle is comprised of a calcium-containing inorganic microparticle which is hardly soluble in water and a biological active substance encapsulated within the microparticle.

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CLAIMS

[Claim(s)]
[Claim 1] a calcium content water poorly soluble inorganic substance particle and the biological active substance enclosed with the interior of the particle concerned -- since -- the becoming drug enclosure inorganic substance particle.
[Claim 2] The drug enclosure inorganic substance particle according to claim 1 characterized by said biological active substance being the protein which has drug effect, the low molecular weight compound which has drug effect, or a gene.
[Claim 3] The drug enclosure inorganic substance particle according to claim 1 characterized by said biological active substance containing 0.0001 to 10% of the weight to said calcium content water poorly soluble inorganic substance.
[Claim 4] The drug enclosure inorganic substance particle according to claim 1 to which the protein which has said drug effect is characterized by being EPO, G-CSF, GM-CSF, thrombopoietin, interferon-alpha, interferon beta, interferon gamma, urokinase, t-PA, IL-11, en BURERU, FGF, EGF and HGF, BDNF, NGF, leptin, NT-3, SOD, an insulin, a human growth hormone, an antibody, and an antigen.
[Claim 5] The drug enclosure inorganic substance particle according to claim 1 to which the low molecular weight compound which has said drug effect is characterized by being blood vessel agonists, such as antiinflammatory drugs, such as non-anti-inflammatory steroid hormone and hydrocortisones, an antimicrobial drug, an anti-gun agent, and a prostagladin, anti-arteriosclerosis medicine, an immunosuppresant, calcitonin, a LHRH derivative, other hypophysis peptide hormone, a vancomycin, tee KOPURANIN, and PTH.
[Claim 6] The drug enclosure inorganic substance particle according to claim 1 to which said calcium content water poorly soluble inorganic substance is characterized by being a calcium carbonate, calcium phosphate, a calcium oxalate, and uric-acid calcium.
[Claim 7] The drug enclosure inorganic substance particle according to claim 1 to which said calcium content water poorly soluble inorganic substance particle is characterized by being a particle with a diameter of 100nm - 200 micrometers.
[Claim 8] The drug enclosure inorganic substance particle according to claim 1 to which said calcium content water poorly soluble inorganic substance particle is characterized by being a particle with a diameter of 10nm - 1,000nm.
[Claim 9] Drug enclosure inorganic substance particle pharmaceutical preparation characterized by consisting of having added the additive which can be received in

galenical pharmacy to the drug enclosure inorganic substance particle according to claim 1.

[Claim 10] Drug enclosure inorganic substance particle pharmaceutical preparation according to claim 9 characterized by said additives which can be received in galenical pharmacy being protein, an acid mucopolysaccharide object, a lactic-acid glycolic-acid polymer, a lactic-acid polymer, a surfactant, a mannitol, antiseptics, and a stabilizing agent.

[Claim 11] Drug enclosure inorganic substance particle pharmaceutical preparation according to claim 9 or 10 characterized by being the gestalt to which drug enclosure inorganic substance particle pharmaceutical preparation according to claim 9 fitted subcutaneous injection, an intramuscular injection, and intravascular injection.

[Claim 12] (1) a calcium salt water solution -- preparing -- (2) -- the manufacturing method of the drug enclosure inorganic substance particle characterized by making a calcium content water poorly soluble inorganic substance particle enclose a biological active substance with this solution by carrying out addition mixing of the biological active substance water solution, and subsequently carrying out addition mixing of (3) carbonates, phosphate, an oxalate, or the urate water solution.

[Claim 13] (1) the water solution of a carbonate, phosphate, an oxalate, or urate -- preparing -- (2) -- the manufacturing method of the drug enclosure inorganic substance particle characterized by making a calcium content water poorly soluble inorganic substance particle enclose a biological active substance with this solution by carrying out addition mixing of the biological active substance water solution, and subsequently carrying out addition mixing of the (3) calcium salt water solution.

[Claim 14] The manufacturing method of the drug enclosure inorganic substance particle according to claim 12 or 13 characterized by adding protein, an acid mucopolysaccharide object, a surfactant, and a mannitol to reaction mixture in order to prevent condensing while the calcium content water poorly soluble inorganic substance particle with which said biological active substance was made to enclose generates.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the gradual-release pharmaceutical preparation using the drug enclosure inorganic substance particle pharmaceutical preparation using the protein which has drug effect, the low molecular weight compound which has drug effect, the calcium content water poorly soluble inorganic substance particle with which the gene was made to enclose, its manufacturing method, and its calcium content water poorly soluble inorganic substance particle, the protein which has drug effect in detail, an antigen, a gene, and the calcium content water poorly soluble inorganic substance particle with which the low molecular weight compound which has drug effect was made to enclose, target gradual-release pharmaceutical preparation, and its manufacturing method. It is related with the pharmaceutical preparation which furthermore enclosed this particle with the matrix which consists of a lactic-acid polymer used for retrogenerative medicine.
[0002] There is a report which used calcium content mineral matter as support of a drug as a Prior art, the front face of the fine crystal of a hydro oxy-apatite is made to support an anticancer agent etc., and the approach of injecting an animal is devised. In addition, the gradual release pharmaceutical preparation using a porous apatite is also considered. However, it is only that the report using a calcium content water poorly soluble inorganic substance particle and patent of those other than calcium phosphate have the patent (JP,07-165613,A, JP,08-027031,A) as collunarium which used the calcium carbonate.
[0003] Moreover, although attaching a drug on the surface of a particle was performed, enclosing a drug with the interior of a calcium content water poorly soluble inorganic substance particle was not performed.
[0004] That is, when forming a calcium content water poorly soluble inorganic substance particle, while the biological active substance was made to live together and the inorganic substance particle was formed, the technique of having made the biological active substance enclose into the inorganic substance particle was not performed.
[0005]
[Problem(s) to be Solved by the Invention] In recent years, protein drugs increased in number by advance of biotechnology. However, if protein drugs are not based on

injection, they cannot be prescribed for the patient, and there are many short things of a half-life. Therefore, the device which lengthens a half-life by the easy approach is needed.
[0006] Moreover, a low-molecular drug, activity protein, a vaccine, and the target of a gene mainly serve as a technical problem more important future still to a macrophage, a reticuloendothelial system organization, neutrophil leucocyte, a vascular endothelial cell, a gun cell, an inflammation part, an infection part, a gun organization, and an arteriosclerosis wall.
[0007] Then, this invention aims at offering the drug enclosure inorganic substance particle from which the gradual-release effectiveness which could stabilize the protein which the pharmaceutical-preparation production approach is easy, and there is stimulative [no], and can apply to the protein which has much drug effect, the low molecular weight compound which has drug effect, and a gene, and has the drug effect concerned, the low molecular weight compound which has drug effect, and the gene, and was excellent, and the target effectiveness are acquired, its manufacturing method, and drug enclosure inorganic substance particle pharmaceutical preparation.
[0008]
[Means for Solving the Problem] the biological active substance with which the drug enclosure inorganic substance particle of this invention was enclosed with the interior of a calcium content water poorly soluble inorganic substance particle and the particle concerned in order to attain said purpose -- since -- it becomes.
[0009] Although the low molecular weight compound or gene which has the protein which the particle which enclosed the biological active substance with the interior of a calcium content water poorly soluble inorganic substance particle is easy to manufacture, and there is stimulative [no], and has drug effect as a biological active substance, and drug effect is used, the protein which has especially drug effect, such as protein which has such drug effect, can be stabilized.
[0010] In case a calcium content water poorly soluble inorganic substance particle is formed, after making an inorganic substance particle form, calling it enclosure that the biological active substance has combined with the interior of the particle concerned in this invention and making an inorganic substance particle form after making a biological active substance live together, the rate of enclosure of a drug produces the effectiveness that it is high and the elution of a drug is gradual, rather than the approach of making an inorganic substance particle drug supporting, by mixing with a drug.
[0011] As protein which has drug effect, erythropoietin (EPO), a granulocyte colony-stimulating factor (G-CSF), A granulocyte-macrophage colony-stimulating factor (GM-CSF), thrombopoietin, Interferon-alpha, interferon beta, interferon gamma, urokinase, An organization plasminogen activator (t-PA), interleukin -11 (IL-11), En BURERU, a fibroblast growth factor (FGF), an epidermal growth factor (EGF), a hepatocyte growth factor (HGF), a brain-derived neurotrophic factor (BDNF), a nerve growth factor (NGF), leptin, and nu -- fatty tuna -- a fin -3 (NT-3), superoxide dismutase (SOD), an insulin, a human growth hormone, an antibody, an antigen, etc. are mentioned. EPO, G-CSF, interferon-alpha, FGF, EGF and HGF, BDNF, NGF, leptin, NT-3, etc. are especially desirable.

[0012] Moreover, it is desirable for the biological active substance to contain 0.0001 to 10% of the weight to said calcium content water poorly soluble inorganic substance. the drug with which a biological active substance furthermore has affinity in calcium -- good -- general -- charge of a positivity [calcium] -- **** -- since it is, a thing with negative charge is desirable.
[0013] As a low molecular weight compound which has drug effect, blood vessel agonists, such as antiinflammatory drugs, such as non-anti-inflammatory steroid hormone and hydrocortisones, an antimicrobial drug, an anti-gun agent, and a prostagladin, anti-arteriosclerosis medicine, an immunosuppresant, calcitonin, a luteinizing hormone releasing hormone (LHRH) derivative, other hypophysis peptide hormone, a vancomycin, tee KOPURANIN, parathyroid hormone (PTH), etc. are mentioned. Especially, antimicrobial drugs, such as an antimicrobial agent and an antifungal agent, an anti-inflammatory agent, an anti-gun agent, blood vessel agonist, etc. are desirable. Moreover, in the case of the low low-molecular drug of calcium affinity, it is good to use the low-molecular drug which carried out covalent bond of the high connectives of calcium affinity, such as a phosphoric acid, to residue by the ester bond etc.
[0014] As a calcium content water poorly soluble inorganic substance, a calcium carbonate, calcium phosphate (an apatite, hydro oxy-apatite, etc.), a calcium oxalate, uric-acid calcium, etc. are desirable.
[0015] this invention persons aim at the target effectiveness to the focus section, and the gradual release effectiveness that a drug is emitted little by little in a body, by enclosing and injecting the interior of the particle with a drug paying attention to a calcium content inorganic substance being the particle of water poor solubility. Namely, with an inflammation part, an infection part, a gun organization, and an arteriosclerosis wall, the gap of several 10 to several 100nm is in a blood vessel wall. Since 10nm - 1,000nm of particles with a magnitude of 10nm - 500nm will be preferably accumulated in the focus through the gap among the particles of this invention The target effectiveness can be produced and the target effectiveness and the gradual release effectiveness of the duplex that phagocytosis is carried out to inflammatory cells and gun cells, such as a macrophage, can be produced.
[0016] Moreover, when the magnitude of the particle concerned is the diameter of 100nm - 200 micrometers, in subcutaneous injection or an intramuscular injection, it is useful as gradual release pharmaceutical preparation.
[0017] Furthermore, big power is demonstrated also to gradual release of the growth factor used for the remarkable playback medicine of an advance in recent years. Moreover, when using for playback medicine, a particle uses a little larger particle as it is, and also it is desirable to enclose with the matrix which consists of a lactic-acid polymer (PLA) etc.
[0018] Production of the last pharmaceutical preparation puts dispersants, such as protein, such as the additive which can be received in galenical pharmacy, i.e., a human serum albumin etc., (HSA), an acid mucopolysaccharide object, a lactic-acid glycolic-acid polymer (PLGA), a lactic-acid polymer, a surfactant, and a mannitol, a stabilizing agent, and antiseptics into the obtained drug enclosure inorganic substance particle, and makes the last pharmaceutical preparation with desiccation or freeze drying. This is suspended with water or buffer solution, and Homo sapiens

is medicated with it so that it may become an isotonicity. Moreover, since semantics which prevents a pain etc., and distribution are made into what was further excellent, suspending and using for hyaluronic acid etc. is also possible. Or it is also possible to make suspension which put in the dispersant etc. the last pharmaceutical preparation as it is.

[0019] Moreover, the last pharmaceutical preparation concerned is the thing of a gestalt suitable for subcutaneous injection, an intramuscular injection, and intravascular injection.

[0020] The manufacture approach of the drug enclosure inorganic substance particle of this invention (1) The water solution of calcium salts, such as a calcium chloride, a calcium bromide, and calcium acetate, is prepared. (2) Addition mixing of the biological active substance water solution is carried out at this solution. Subsequently (3) sodium carbonates, Carbonates, such as potassium carbonate, sodium phosphate, dibasic sodium phosphate, It is making a biological active substance enclose with a calcium content water poorly soluble inorganic substance particle by carrying out addition mixing of the water solution of urate, such as oxalates, such as phosphate, such as potassium phosphate, a sodium oxalate, and a potassium oxalate, or sodium urate, and a uric-acid potassium.

[0021] moreover, the water solution of (1) carbonate, phosphate, an oxalate, or urate -- preparing -- (2) -- it is making a calcium content water poorly soluble inorganic substance particle enclose a biological active substance with this solution by carrying out addition mixing of the biological active substance water solution, and subsequently carrying out addition mixing of the water solution of calcium salts, such as (3) calcium chlorides, a calcium bromide, and calcium acetate.

[0022] Furthermore, in order to prevent condensing while the calcium content water poorly soluble inorganic substance particle with which the biological active substance was made to enclose generates, In order to prevent adding protein, an acid mucopolysaccharide object, a surfactant, and a mannitol to reaction mixture, and that the calcium content water poorly soluble inorganic substance particle which made the biological active substance enclose condenses in the living body, Moreover, in order to avoid phagocytosis in the living body, it is desirable to add protein, an acid mucopolysaccharide object, a lactic-acid glycolic-acid polymer, and a lactic-acid polymer to a drug enclosure inorganic substance particle.

[0023] In addition, when mixing two or the inorganic substance beyond it, it can be desirable to mix stirring before and behind pH7, and it can make inorganic substance concentration, drug concentration, stirring speed, reaction time, and temperature adjust grain size in a manufacturing method. Although only a 1 micrometer - 100 micrometers particle is made in the usual stirring by the stirrer, a 10nm - 100 micrometers particle can also be made by increasing the stirring force further with a vortex, the poly TRON, a supersonic wave, etc. Moreover, as for a water solution, it is desirable to use the buffer solution which considers as neutrality as much as possible, makes ionic strength as low as possible, and is not combined with calcium.

[0024]

[Example] The example of this invention and the example of a trial are described

below.
(Example 1) It mixed, stirring 5M CaCl2(Wako) 650microl and 1mg/ml EPO(Chugai Pharmaceutical)125microl. Added 1M Na2CO3 (Wako)2.5ml there, the line made CaCO3 particle generate stirring for 10 minutes, and EPO was made to enclose with the interior of CaCO3 particle. H20 5ml was added to this, centrifugal [of 2,000rpm and 5min] was applied, and supernatant liquid was removed. It poured [at the obtained dregs] distributively in H20 6.25ml, in addition 1.5ml test tube four per every ml. Four were applied to centrifugal [of 2,000rpm and 5min], and supernatant liquid was removed. Inside, in order for two to carry out the quantum of the EPO enclosed with CaCO3 particle, ELISA measurement was presented with them. 1M Na2CO3 0.9ml, in addition stirring were performed, two remaining were put, and EPO combined with the front face was separated. After putting for 10 minutes, centrifugal was applied, and supernatant liquid was removed. The same activity was done again and EPO which separated by Na2CO3 processing was removed in centrifugal. The dregs which remained were carried out to ELISA measurement. EPO enclosed into the CaCO3 particle dissolved CaCO3 particle with the hydrochloric acid, separated EPO, and was measured using ELISA.
[0025] (Example 1 of a trial) after mixing with 5M CaCl2 650microl and 1M Na2CO3 2.5ml previously, producing CaCO3 particle and washing in H20 -- 1mg/-- the sample was prepared [ml EPO 125micro1]. H20 5ml was added to this, centrifugal [of 2,000rpm and 5min] was applied, and supernatant liquid was removed. EPO which performs the completely same actuation as an example 1 below, and exists in a CaCO3 particle front face was measured. Unlike the sample which the result made combine EPO with the particle front face of the example 1 of a trial as shown in drawing 1, with the particle sample by the method of enclosing the example 1 based on this invention approach, EPO hardly separated by Na2CO3 processing, but it became clear that all are mostly enclosed in a particle.
[0026] (Example 2) 5M CaCl2650microl and 500microg/ml It mixed stirring G-CSF (Chugai Pharmaceutical) 250microl. 1M Na2CO3 2.5ml was added there, and the line prepared CaCO3 particle for stirring for 10 minutes. H20 5ml was added to this, centrifugal [of 2,000rpm and 5min] was applied, and supernatant liquid was removed. It poured [at dregs] distributively four per every ml in H2O 6.25ml, in addition 1.5ml test tube. Four were applied to centrifugal [of 2,000rpm and 5min], and supernatant liquid was removed. Inside, in order for two to carry out the quantum of G-CSF enclosed with CaCO3 particle, ELISA measurement was presented with them. 1M Na2CO3 0.9ml, in addition stirring were performed, two remaining were put, and the activity which separates G-CSF combined with the front face was done. After putting for 10 minutes, centrifugal was applied, and supernatant liquid was removed. The same activity was done again and G-CSF which separated by Na2CO3 processing was removed in centrifugal. The dregs which remained were carried out to ELISA measurement. G-CSF enclosed into the CaCO3 particle dissolved CaCO3 particle with the hydrochloric acid, and measured G-CSF which separated using G-CSF ELISA (IBL).
[0027] (Example 2 of a trial) It mixed with 5M CaCl2 650microl and 1M Na2CO3 2.5ml previously, and CaCO3 particle was produced, and after washing in H2O, 500microg/the sample ml G-CSF 250microl Added was prepared. H20 5ml was

added to this, centrifugal [of 2,000rpm and 5min] was applied, and G-CSF in supernatant liquid was removed. G-CSF which performs the completely same actuation as an example 2 below, and exists in CaCO3 particle was measured. Unlike the sample which the result made combine G-CSF with the particle front face of the example 2 of a trial as shown in drawing 2, with the sample by the method of enclosing an example 2, although some separated by Na2CO3 processing in G-CSF, it was proved that most amount is enclosed in a particle.
[0028] (Example 3) It mixed with 375micro (Hydrocorton soluble, Banyu Pharmaceutical) of pharmaceutical preparation I containing 5M CaCl2650microl and 5% of phosphoric-acid hydrocortisone [HyC (Phos.)], stirring in a vortex. 1M Na2CO32.5ml was added there and the line prepared CaCO3 particle for stirring for 10 minutes. H2O 5ml was added to this, centrifugal [of 2,000rpm and 5min] was applied, and HyC (Phos.) which is not enclosed with CaCO3 particle was removed. This part was carried out to ELISA measurement. Moreover, H2O 6.25ml, in addition its 1ml were moved to dregs at 1.5ml test tube. The hydrochloric acid was added to this, CaCO3 particle was melted completely, and it carried out to ELISA measurement. Before ELISA measurement, it mixed with the liver homogenate liquid of a mouse at a rate of 1:1, and it incubated at 37 degrees C for 2 hours, HyC (Phos.) was hydrolyzed, it was referred to as free HyC, and measurement was presented. From the mouse, liver homogenate liquid extracted liver, added H2O 5ml to a part for a piece object, and it carried out the homogenate by the poly TRON. Centrifugal [of 15,000rpm and 5min] was applied, the supernatant liquid was collected, and this was made into liver homogenate liquid.
[0029] (Example 3 of a trial) after mixing with 5M CaCl2 650microl and 1M Na2CO3 2.5ml previously, producing CaCO3 particle and washing in H2O -- HyC(Phos.) 375micro -- I in addition, the sample was prepared. H2O 5ml was added to this, centrifugal [of 2,000rpm and 5min] was applied, and HyC (Phos.) which has not been combined with CaCO3 particle was removed. HyC which performs the completely same actuation as an example 3 below, and exists in CaCO3 particle was measured. As the result was shown in drawing 3, as for the amount of HyC(s) combined thru/or enclosed with the particle among the total amounts of HyC(s) in suspension, it was proved that the sample of the example 3 of a trial is about 20%, and low-molecular drugs are also enclosed for the sample of an example 3 into CaCO3 particle 70% or more.
[0030] (Example 4) It mixed, stirring 5M CaCl2650microl and 500microg/ml G-CSF 250microl. 1M Na2CO3 2.5ml was added there, and the line prepared CaCO3 particle for stirring for 10 minutes. H2O 5ml was added, centrifugal [of 2,000rpm and 5min] was applied, and supernatant liquid was removed. H2O 12.5ml, in addition 1ml were moved to dregs at 2.0ml test tube. Centrifugal [of 2,000rpm and 5min] was applied to this, and supernatant liquid was carried out to ELISA measurement. 1ml (pH7.2) of BSA/Tris-HCl was added to dregs 1%, and it shook at the room temperature. Centrifugal [of 2,000rpm and 5min.] was applied every 24 hours, 1ml (pH7.2) of BSA/Tris-HCl was again added for supernatant liquid to recovery and dregs 1%, and this activity was done for seven days. It was made to dissolve with a hydrochloric acid and the last dregs carried out to ELISA measurement. ELISA Kit of IBL was used for measurement of G-CSF, and G-CSF

was enclosed. Total (accumulation) showed the amount of G-CSF emitted from CaCO3 pharmaceutical preparation. Moreover, as contrast, it mixed with BSA/Tris-HCl(pH7.2) 5ml with 100microg/ml G-CSF 30micro1 within the test tube 1% at coincidence, and shook at the room temperature. The part was extracted every 24 hours, the amount of G-CSF was measured in ELISA, and the stability of G-CSF in a room temperature was examined. The result was shown in drawing 4. It became clear that G-CSF is gradually emitted for seven days or more by the emission trial from CaCO3 particle of G-CSF. Although it is quite low compared with the amount of G-CSF (10microg) which was set to 0.7microg and used when the amount of G-CSF of the sediment of seven days after is totaled, this is considered based on the instability of G-CSF eluted in buffer solution. The instability of G-CSF in buffer solution is clear also from the deactivation curve (a white round head, broken line) when leaving a free G-CSF solution at a room temperature. It became clear that the direction of G-CSF in CaCO3 particle is farther [than G-CSF in a solution] stable.

[0031] (Example 5) It mixed, stirring 5M CaCl2650microl and 1 mg/mlEPO 125micro1. 1M Na2CO3 2.5ml was added there, and the line prepared CaCO3 particle for stirring for 10 minutes. H2O 5ml was added, centrifugal [of 2,000rpm and 5min] was applied, and EPO in supernatant liquid was removed. H2O 3ml, in addition 1ml were moved to dregs at 1.5ml test tube. Centrifugal [of 2,000rpm and 5min] was applied and supernatant liquid was removed completely. It stirred by having added chondroitin-sulfate-A sodium (CS-A, Wako) 0.3ml and 5%Mannitol (Wako)2.1ml 2%, and the sample was adjusted. The sample with which 1mg/ml EPO 30microl and 5%Mannitol 1.77ml were made to mix as control was also prepared. 200microl administration of these was done for five months at the muscles of the C3 H/helium mouse of an age male, eye socket blood collecting was performed 1, 2, 3, and day by day [4] for after [administration] 4 hours, and the blood drug concentration of EPO was measured in ELISA. Although the result was shown in drawing 5, when intramuscular injection of the EPO-CaCO3 pharmaceutical preparation on which CS-A was made to act in the semantics which prevents condensation and organization association was carried out, the gradual release effectiveness was acquired by in vivo.

[0032] (Example 6) It mixed with 375micro of pharmaceutical preparation I containing 5M CaCl2650microl and 5% of HyC (Phos.), stirring. It stirred by having added 1M Na2CO32.5ml there, and CaCO3 particle was prepared. In order to prevent joining together by the produced CaCO3 particles, 3.525ml of CS-A was added 2%, and stirring was performed for 10 minutes. H2O 5ml was added, centrifugal [of 2,000rpm and 5min] was applied, and HyC (Phos.) which has not been combined with CaCO3 particle was removed. 1.397ml of 1%CS-A/5% Mannitol (s) was added to dregs, and it carried out to rat administration. Moreover, as control, it mixed with HyC(Phos.)300microl, and 80/0.5% BSA of 0.1%Tween(s) / 5% Mannitol 818microl, it was adjusted, and 500microl was administered intravenously to the Wistar rat of an age male 10 round. Blood was extracted from the mouse after [administration] 10 minutes and, and 1 hour after, and the spleen was extracted 48 hours after. In addition, blood made 37 degrees C of mouse liver homogenate liquid act for 2 hours, in order to decompose into HyC HyC (Phos.)

which remains with phosphoric ester. The homogenate of the spleen was carried out enough, and after it did the activity which crushes a cell, it made mouse liver homogenate liquid act, was extracted by DMSO after that, and performed ELISA measurement. The value of the rat non-prescribed a medicine for the patient was lengthened, and it considered as the amount of HyC(s). ELISA used CortisolEIA (IBL). As the result was shown in drawing 6 , in the case of CaCO3 particle pharmaceutical preparation, it turned out that many targets of the HyC are carried out to the spleen which has a gap in a blood vessel wall like an inflammation blow hole or a gun organization.

[0033] (Example 7) It mixed with 375micro of pharmaceutical preparation I containing 5M CaCl2650microl and 5% of HyC (Phos.), stirring. It stirred by having added 1M Na2CO3 2.5ml there, and CaCO3 particle was prepared. In order to prevent joining together by the produced CaCO3 particles, 3.525ml of CS-A was added 2%, and stirring was performed for 10 minutes. H2O 5ml was added, centrifugal [of 2,000rpm and 5min] was applied, and HyC (Phos.) which has not been combined with CaCO3 particle was removed. 12.5ml of Mannitol(s) was added to dregs 1%CS-A / 5%, and the sample was adjusted. intraperitoneal [of a mouse] -- 10% proteose peptone 2.0ml -- it injected and the extraction peritoneal macrophage was extracted three days after. It adjusted to 5X105 cells/ml, and 0.1ml of this cell suspension was put into 24wells plate into which the cover glass was put, it was cultivated, and the macrophage was made to adhere. After removing the cell not adhering and replacing culture medium, HyC (Phos.) enclosure CaCO3 0.375.ml was added, and it incubated at 37 degrees C. 24 hours after, the cover glass was taken, the Giemsa stain was carried out after the air dried, and the situation of the phagocytosis of CaCO3 particle was observed under the microscope. As the result showed drawing 7, the image HyC (Phos.) enclosure CaCO3 particle is carrying out [the image] phagocytosis clearly in a macrophage was accepted.

[0034] (Example 8) It mixed, stirring 5M CaCl22.6ml and 500microg/ml G-CSF1ml. 1M Na2CO310ml was added there and the line prepared CaCO3 particle for stirring for 10 minutes. H2O 20ml was added, centrifugal [of 2,000rpm and 5min] was applied, and G-CSF in supernatant liquid was removed. H2O 3ml was added to this, and it moved to vial tubing. This was applied to freeze drying and powder was adjusted. It mixed with 50mg of G-CSF enclosure CaCO3 particles, PLGA2.61g, and dichloromethane 3ml. It mixed stirring this in a zinc acetate solution 0.1% polyvinyl alcohol / 0.7%. After 3-hour stirring, it applied to centrifugal [of 1,000rpm] and dregs were obtained. By H2O, through and 20% Mannitol 0.7microl were added, the dry freeze of the 250-micrometer filter was carried out after washing, and the sustained release drug was obtained.

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is drawing showing the amount of EPO(s) in the constituent of an example 1 and the example 1 of a trial.
[Drawing 2] It is drawing showing the amount of G-CSF in the constituent of an example 2 and the example 2 of a trial.
[Drawing 3] It is drawing showing the amount of HyC(s) (Phos.) in the constituent of an example 3 and the example 3 of a trial.
[Drawing 4] It is drawing having shown the emission nature of G-CSF from the constituent of an example 4, and is drawing doubling and showing the stability of G-CSF in the buffer solution considered as contrast.
[Drawing 5] It is drawing showing EPO blood-drug-concentration transition after the mouse intramuscular injection of the pharmaceutical preparation of an example 5.
[Drawing 6] It is drawing showing that the pharmaceutical preparation of an example 6 compares with control after mouse intravenous injection, and shifts to a spleen intentionally.
[Drawing 7] The pharmaceutical preparation of an example 7 is drawing which observed being incorporated by the macrophage.

[Translation done.]

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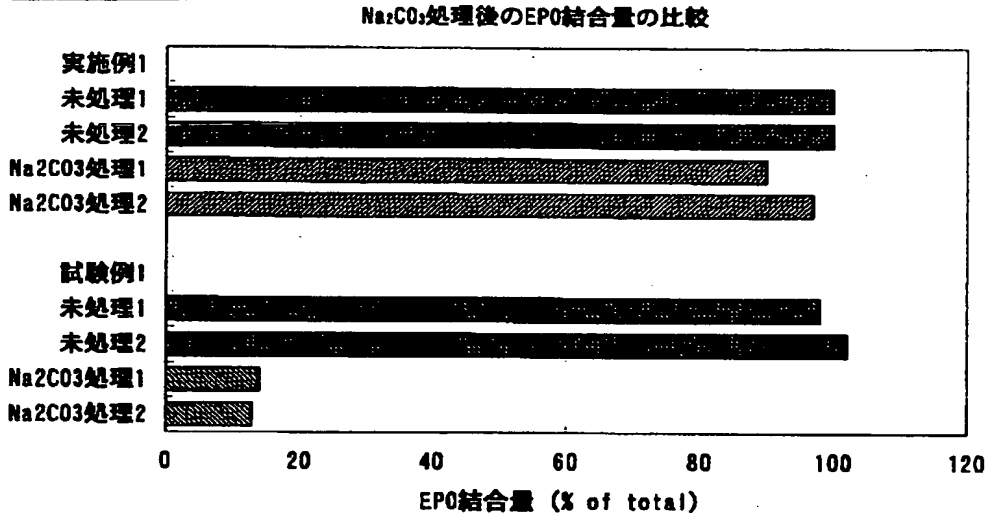
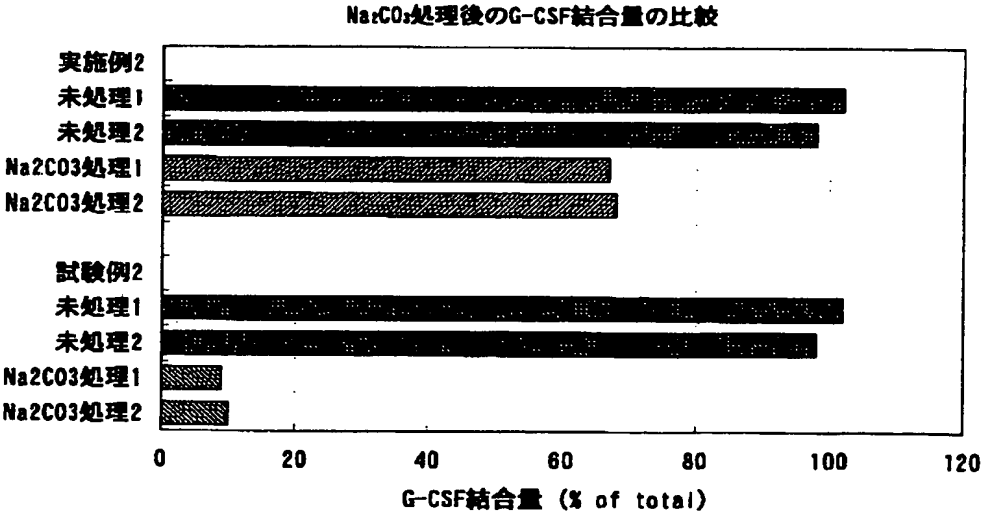
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DRAWINGS

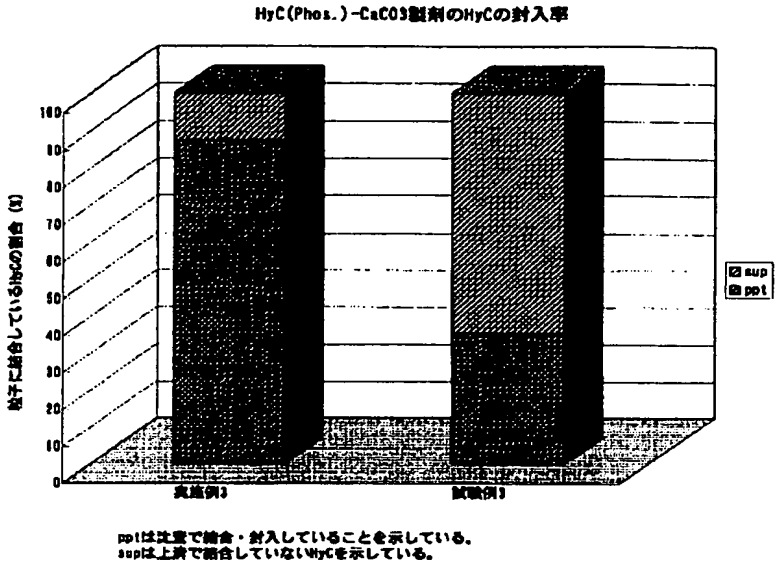
[Drawing 1]



[Drawing 2]



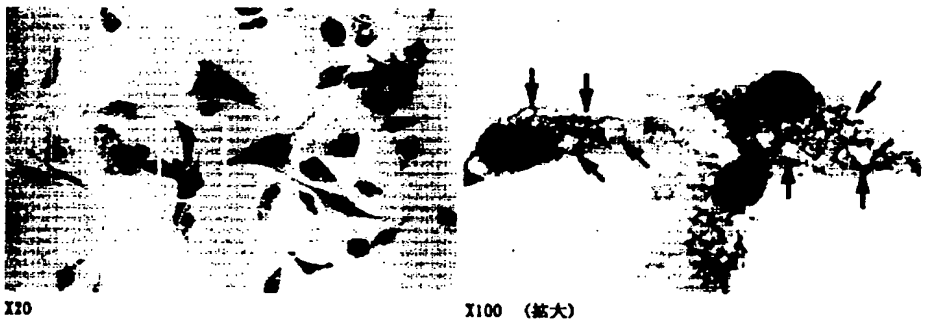
[Drawing 3]



pptは沈査で結合・封入していることを示している。
supは上清で結合していないHyCを示している。

[Drawing 7]

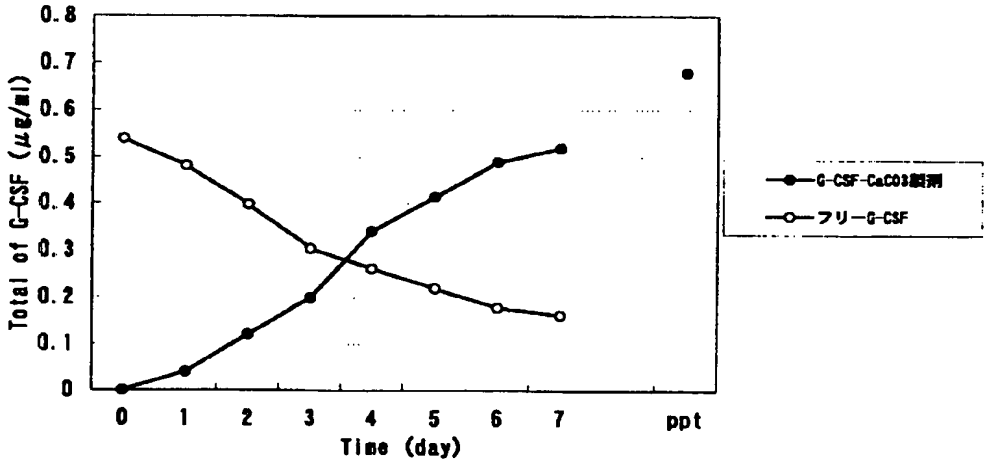
腹腔マクロファージによる HyC(Phos.)封入 $CaCO_3$ 微粒子の取り込み



X10　　X100 （拡大）

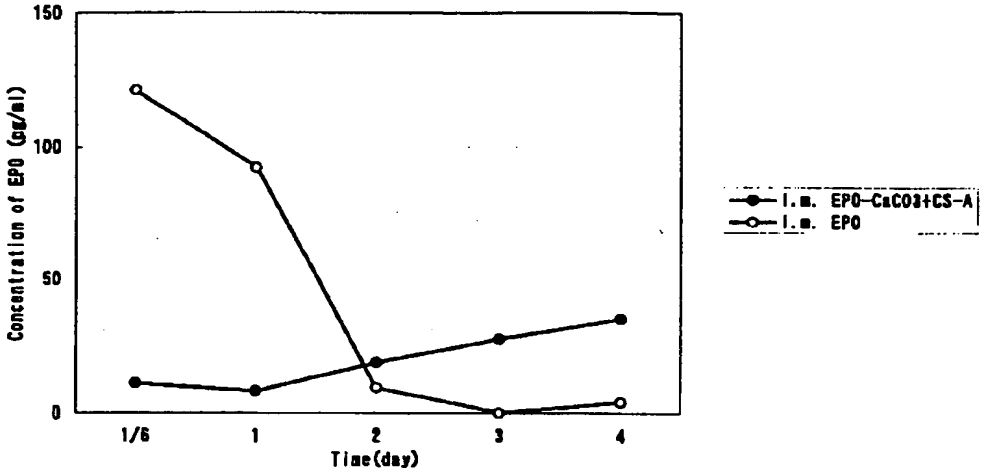
[Drawing 4]

G-CSF (in vitro)の徐放実験とG-CSFの安定性（室温）



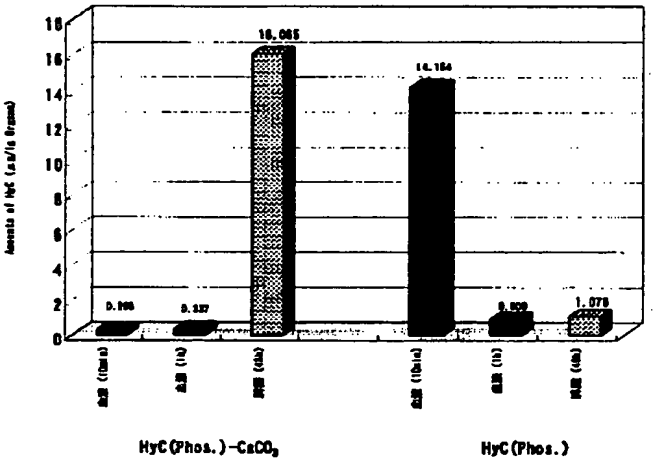
[Drawing 5]

EPO-$CaCO_3$製剤投与後のEPO血中濃度の推移（マウス、in vivo）



[Drawing 6]

HyC-$CaCO_3$製剤とHyC(Phos.)投与後のHyCの血漿濃度（ラット）



[Translation done.]